

## Genetic effects on task performance, but not on age polyethism, in a swarm-founding eusocial wasp

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**Abstract.** Division of labour among workers in insect societies often includes two major components: age-related changes in behaviour (age polyethism) and specialization in task performance. The aim of this study was to test whether similarity in inside-nest task performance and in rate of age polyethism correspond to genetic similarity among nestmates in the polygynous eusocial wasp *Polybia aequatorialis*. Behavioural data were collected on marked, known-age workers from three source colonies introduced into two observation colonies in the field. Genetic similarity among workers was assessed by quantifying sharing of random amplified polymorphic DNA (RAPD) marker alleles. Workers were categorized by whether they engaged in nest cleaning as an indicator of individual differences in inside-nest task performance. Within source colonies, workers that performed nest-cleaning tasks were more genetically similar to each other than they were to workers not performing these tasks. Workers also differed in their rates of passage through the age-related task sequence, but no association was found between sharing of RAPD marker alleles and rate of age polyethism. These results accord with earlier studies demonstrating flexibility in age polyethism in swarm-founding wasps, and with findings that worker genotypic variability corresponds to specialization in task performance in *P. aequatorialis*. *Polybia* spp. workers rarely switch among tasks, even in response to changes in colony conditions, and workers' genotypes may constrain flexibility in task performance at the individual level. Conversely, colonies may accrue benefits from having genotypically diverse worker forces, which could favour the maintenance of polygyny in swarm-founding wasps.

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Workers in eusocial insect colonies often manifest a highly organized division of labour, particularly in those species where adult colony populations reach or exceed several hundred individuals (Wilson 1971). Division of labour among workers often includes two independent components. First, individuals may change the set of tasks they perform as they age, a pattern referred to as age polyethism. Workers typically perform tasks inside the nest following adult emergence, later moving out to the nest exterior, then leave the nest to forage when older (Wilson 1976; Seeley 1982; O'Donnell & Jeanne 1992a). Although nearly all workers follow this sequence, nestmates can vary greatly in the rate of age polyethism. For example, in the wasp, *Polybia occidentalis*, the age of first foraging ranged from 5 to 40 days, with an

average of 19 days (O'Donnell & Jeanne 1992a). Second, workers can specialize on a subset of the tasks performed by their age cohort. *Polybia* spp. foragers specialized by collecting one of the four materials (prey, nectar, wood pulp and water) gathered by their colonies (Jeanne 1986; O'Donnell 1996). In *P. occidentalis* specialization in material collection often persisted throughout foragers' life spans (O'Donnell & Jeanne 1992a). Although less attention has been devoted to the tasks performed early in life than to foraging, social insect workers can also specialize in tasks that are performed inside the nest. For example, most of the corpse removal or undertaking in honey bee, *Apis mellifera*, colonies is performed by a small subset of the worker force (Visscher 1983).

Individual behavioural differences are the basis of division of labour among workers. The mechanisms underlying these differences are recognized as a key to understanding the organization, and

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ultimately the evolution, of insect colony behaviour (Page & Robinson 1991; Robinson 1992). To understand how insect societies function, it is first necessary to establish the degree to which individual worker behaviour is flexible, and to determine the nature of possible constraints on behavioural flexibility, which may include genetic effects.

Page & Robinson (1991) suggested that genetic differences among workers can be a component of the division of labour. Genetic diversity among workers within insect colonies derives from multiple mating by queens (polyandry), production of offspring by multiple queens (polygyny) and genetic recombination in heterozygous queens. Recent advances in DNA marker techniques for assessing genetic structure within colonies have provided the means to measure associations of genetic variation with division of labour among workers, or polyethism (Page et al. 1995b; O'Donnell 1996). Evidence is accumulating for genetic effects on polyethism in many social insect taxa (honey bees: Fewell & Page 1993; Page et al. 1995b; ants: Stuart & Page 1991; Snyder 1993; wasps: O'Donnell 1996). Genetic effects on worker behaviour are of interest because knowledge of the mechanisms of the division of labour will permit better predictions of colony-level behaviour, and of the selective forces that shape colony genetic structure. If division of labour is based in part on genetic differences, then an individual's genotype may constrain its ability to perform different tasks. Conversely, colonies with a diverse array of genotypes may have a more efficient division of labour (Crozier & Consul 1976) or greater phenotypic plasticity, as has been suggested for honey bees (Fuchs & Schade 1994; Page et al. 1995a).

The goal of this study was to test for associations of genetic similarity among workers with two components of the division of labour (age polyethism and differences in task performance) in the eusocial epiponine wasp, *Polybia aequatorialis*. Genetic effects on age polyethism and performance of inside-nest tasks have been demonstrated in honey bees (Rothenbuhler 1964; Calderone & Page 1996). Epiponine wasps are an important group for comparison with honey bees (which have single polyandrous queens; Estoup et al. 1994). Genotypic diversity within epiponine worker forces results primarily from polygyny, or the presence of multiple egg-laying queens

(Richards 1978; Jeanne 1991). Recent data suggest that epiponine queens mate with a single male (Goodnight et al. 1996). Polygyny is likely to be the principal source of genetic diversity within colonies, although recombination of queens' genomes can also contribute to genetic differences among workers. Earlier work on *P. aequatorialis* demonstrated significant associations within colonies between genetic similarity (as indicated by the sharing of random amplified polymorphic DNA (RAPD) marker bands) and foraging task specialization (O'Donnell 1996). Foragers specialized by collecting predominantly one of the four materials (insect prey, nectar, wood pulp and water) gathered by their colonies, and foragers collecting the same material were significantly more likely to share RAPD marker alleles. Genetic effects on inside-nest task performance and age polyethism have not been quantified in epiponine wasps.

### Subject Species

*Polybia aequatorialis* is a swarm-founding eusocial wasp (Vespidae: Epiponini). *Polybia* spp. colonies are housed in enclosed nests with a single entrance opening and several layers of comb (see Wenzel 1991 for diagrams of nest architecture). At the study site in Monteverde, Puntarenas Province, Costa Rica (10°18'N, 84°49'W), *P. aequatorialis* colonies usually comprise several hundred to a few thousand adult females (personal observation; O'Donnell, in press). Epiponine wasp colonies of this size are typically polygynous (Richards 1978; Jeanne 1991). Because queens are not genetically identical, and probably mate with unrelated males (J. Strassmann, personal communication), polygyny increases genotypic variability (lowers genetic relatedness) within colonies. *Polybia* spp. workers, including *P. aequatorialis*, exhibit well-developed age polyethism and marked specialization on foraging tasks (Jeanne et al. 1988; O'Donnell 1996). Adult workers reared from combs of foreign nests are readily accepted into observation colonies within 24 h of emergence (eclosion) and can be marked for individual recognition with paint pens.

## METHODS

### Subject Colonies and Workers

I conducted field work in December 1995 and January 1996 (early dry season) at approximately

1300 m elevation in Monteverde. I moved two *P. aequatorialis* colonies to an observation site sheltered from direct sun and rain. I transported the colonies at night to ensure that their entire adult populations were present, and left them undisturbed for at least 5 days prior to further manipulations. Observation colonies resumed active foraging on the day after being moved. I collected brood combs from three additional *P. aequatorialis* colonies as sources of subject workers:  $N=49$  from source colony A, 70 from source colony B, and 190 from source colony C. All newly emerged adults were removed from these combs daily and marked with paint pens in a numerical code for individual identification. The large numbers of adults emerging on some days (maximum of 33 adults from source colony A, 16 adults from source colony B and 72 adults from source colony C) and the fact that workers emerged from different combs on the same day (two combs from source colony A, five combs from source colony B and two combs from source colony C) suggest that multiple queens had laid the eggs contributing to each source colony's cohort of workers. I have observed several queens laying eggs simultaneously on *P. occidentalis* combs. I introduced marked adults into the two observation colonies daily on 10 consecutive days preceding and during behavioural observations. I introduced workers from source colonies A and B into one observation colony and workers from source colony C into the other. The original nesting locations of all source and observation colonies were separated by more than 300 m, greater than distances most epiponine wasp swarms have been observed to travel at other sites (Jeanne 1975; West-Eberhard 1982; personal observation). Distances traversed by *P. aequatorialis* swarms have not been quantified. It is not known whether any of the subject colonies were closely related, for example, as daughter colonies. All introduced workers were apparently accepted into the observation colonies.

### Behavioural Observations and Worker Collections

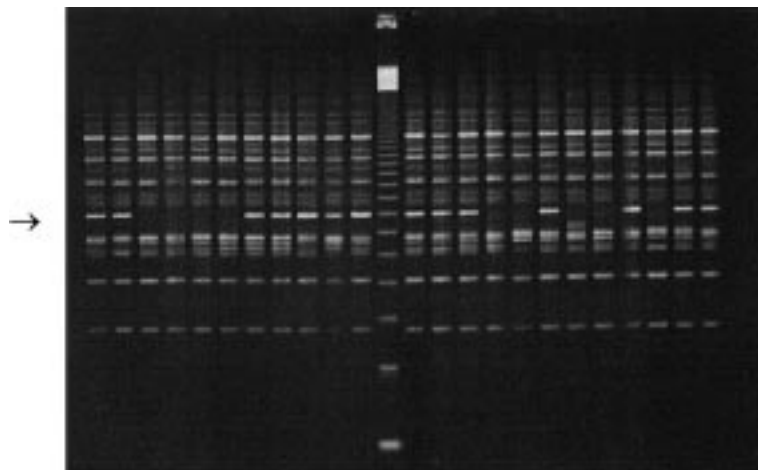
I conducted behavioural observations daily from 17 to 27 January 1996. Behaviour of marked workers was recorded by an observer seated in front of each observation colony. Continuous observation sessions lasted for 2 h each starting at 0800 hours and again at 1330 hours local time.

Scan samples of all workers visible on the nest surface and in the nest entrance were conducted every 10 min. Acts recorded during scans followed a list of behavioural acts developed for *P. occidentalis* (O'Donnell & Jeanne 1993). All occurrences of foraging behaviour were also recorded by noting the identity and time of arrival or departure of marked workers. Acts performed by workers were grouped into task sets based on where they were performed: inside the nest (inside-nest), outside the nest entrance on the envelope (outside-nest) and foraging tasks (off-nest). Inside-nest tasks classified as nest-cleaning behaviour included regurgitating water from inside the nest and carrying or dropping solid waste from inside the nest. Workers flying from inside the nest with solid debris were counted as nest-cleaners rather than as foragers.

After behavioural observations were completed (on 27 January 1996), all adults were collected at night by placing each observation nest into a plastic bag with ether-soaked cotton. Marked adults were sorted out, immediately placed into cold 100% ethanol, and stored at  $-20^{\circ}\text{C}$  until DNA extraction. Unmarked adults belonging to the observation colonies were placed in fixative (Kahle's solution; Barbosa 1974) for later counting.

### Genetic Similarity among Workers

I extracted genomic DNA from marked adult wasps following a standard phenol:chloroform extraction protocol (Sambrook et al. 1989). DNA samples were stored frozen at  $-70^{\circ}\text{C}$  until they were used in polymerase chain reactions. I generated RAPD markers (detailed protocol in Hunt & Page 1995; O'Donnell 1996) using 13 ten-base DNA primers (Operon Technologies, Inc.) which amplify variable markers in the *P. aequatorialis* subject population (O'Donnell 1996). DNA sequences for the primers used, and the sizes of variable RAPD markers, are given in the Appendix. Polymerase chain reaction products were separated on gels of 1% agarose/0.7% Synergel (Diversified Biotech) exposed to a current of 100 V for 5 h. Following electrophoresis, DNA in the gels was stained with ethidium bromide. The gels were then exposed to UV light on a transilluminator and photographed. RAPD marker alleles were scored from the photographs (Fig. 1), and all markers were scored blind to



**Figure 1.** Presence/absence RAPD marker resulting from polymerase chain reaction (PCR) amplification (Operon primer OPG-09) of DNA from 23 *Polybia aequatorialis* workers from source colony A. Each vertical lane contains RAPD-PCR products from a single worker; the twelfth lane from the left is a DNA fragment size marker ladder comprising fragments in multiples of 123 base pairs. DNA migrated from top to bottom in the photograph; smaller fragments travelled further. The variable marker locus (indicated by the arrow at left) co-migrated with the seventh from smallest ladder band, and is approximately 861 ( $7 \times 123$ ) base pairs long. From the left, individuals in positions 1, 2, 7 to 14, 17, 20, 22 and 23 were scored as present; the remainder was scored as absent. As was typical of all RAPD primers used in this study, numerous additional bands (loci) amplified that were invariant across individuals. Invariant bands served as an internal check of PCR quality.

worker identity. Protocols for all laboratory procedures are available from the author upon request.

I scored 24 RAPD marker loci for each source colony. Some marker loci were not variable within some source colonies, so the number of variable informative markers used in analysis ranged from 20 to 22 per source colony. All markers were scored as presence/absence polymorphisms except one fragment-length polymorphism (with two alleles) in source colonies A and B. RAPD loci that vary as presence/absence polymorphisms produce dominant marker alleles; heterozygous individuals cannot be distinguished from those homozygous for the present allele (Lynch & Milligan 1994). Although true genotypes cannot always be distinguished at a given locus, sharing of presence/absence RAPD markers provides information on genetic similarity among individuals (Fondrk et al. 1993; Dreller et al. 1995; Hasegawa 1995; O'Donnell 1996; Reichardt & Wheeler 1996). Statistical analyses tested whether individuals that were more genetically similar were also more similar in behaviour.

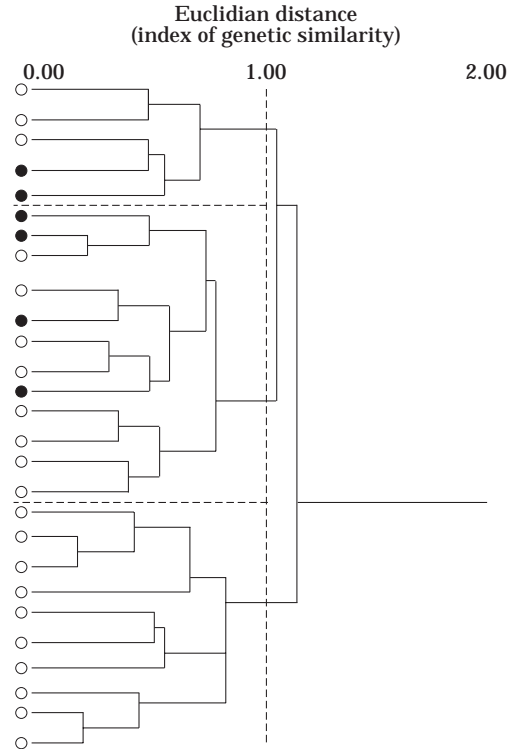
### Statistical Analyses

I used the survival analysis Wilcoxon test (SAS Institute 1985) to assess whether individuals that shared RAPD alleles had more similar rates of age polyethism. In survival analysis, the Wilcoxon test is used to analyse differences in the distribution of age of occurrence of events (here, the onset of performance of outside-nest and off-nest tasks) among groups (here, individuals sharing RAPD marker alleles). In this study, the workers that had not performed outside-nest or off-nest tasks by the end of the observations were included in the survival analysis. This was done by entering their ages at the end of the study as censored values in the distributions of age of first performance of those tasks. Censoring of some data points was used to correct for the fact that the study terminated before the behavioural acts of interest had been performed by some individuals. Censoring makes survival analysis a powerful tool in analysing temporal distributions because it increases the power of statistical tests and prevents biases associated with excluding individuals that have not performed the behaviour of interest from the

analysis. See Pyke & Thompson (1986) for further discussion of survival analysis and censoring.

I performed the survival analysis Wilcoxon test independently for each of the variable RAPD marker loci in each source colony. Because statistical associations between RAPD alleles and behaviour could occur by chance at some loci, I analysed the significance of the overall association between RAPD allele sharing and rate of age polyethism by summing the values of the Wilcoxon test statistics and degrees of freedom within source colonies. This pooling method is similar to that used by O'Donnell (1996) to test for associations of shared RAPD alleles with forager specialization. The pooled test assumes statistical independence (i.e. no genetic linkage) among marker loci. If there were genetic linkage among RAPD marker loci, the probability of finding significant overall associations of RAPD allele sharing and behaviour could be inflated, making a spurious positive result (type I error) more likely (O'Donnell 1996).

To test for genetic effects on task performance, I identified clusters of genotypically similar workers following the method of Fondrk et al. (1993). To delimit the clusters of workers, I subjected the matrices of alleles for all RAPD markers and all individuals to hierarchical cluster analysis (SYSTAT 1992) using normalized Euclidean distance and Ward's method of linkage. Individuals that shared more RAPD alleles clustered together (Fig. 2). This clustering algorithm has been shown to elucidate genotypic structure due to multiple paternity within honey bee and ant colonies (Fondrk et al. 1993; Hasegawa 1995) and was used to demonstrate genotype effects on foraging behaviour in a feral honey bee colony (Dreller et al. 1995). The causes of genotypic similarity among workers in the present study are unknown, because the social and genetic history of the source colonies prior to collection of their combs was not determined. Relatedness due to shared maternity presumably contributed to workers clustering near each other. Cluster analysis generates dendrograms representing genotypic similarity among workers. From the dendrograms, I delimited clusters of genotypically similar workers by selecting as cut-offs those branches at 50–75% of the total genetic distance in the dendrogram that yielded from three to five clusters (Fig. 2). All workers joined by nodes above the selected branches belonged to the same cluster.



**Figure 2.** Dendrogram representing patterns of genetic similarity among *Polybia aequatorialis* workers from source colony A. Euclidian distance among workers was calculated based on sharing of RAPD marker alleles at 22 variable marker loci. The clusters of genotypically similar workers that were used in the analysis of the relationship between genetic similarity and task performance are separated by the dashed lines. Individual workers are represented by open circles if they performed nest-cleaning tasks and by filled circles if they did not engage in nest-cleaning.

The choice of cluster-defining branches was performed blind to worker identity.

As an indicator of differences in task performance, I categorized workers by whether they had performed tasks associated with cleaning the nest (removing solid and liquid wastes). For each source colony, I tested whether the probability of performing nest-cleaning tasks was equal among genetic clusters with the likelihood ratio contingency test, which is less biased than the standard chi-square test when expected values in some cells of the contingency table are small (Lewontin & Felsenstein 1965; Fienberg 1989).

## RESULTS

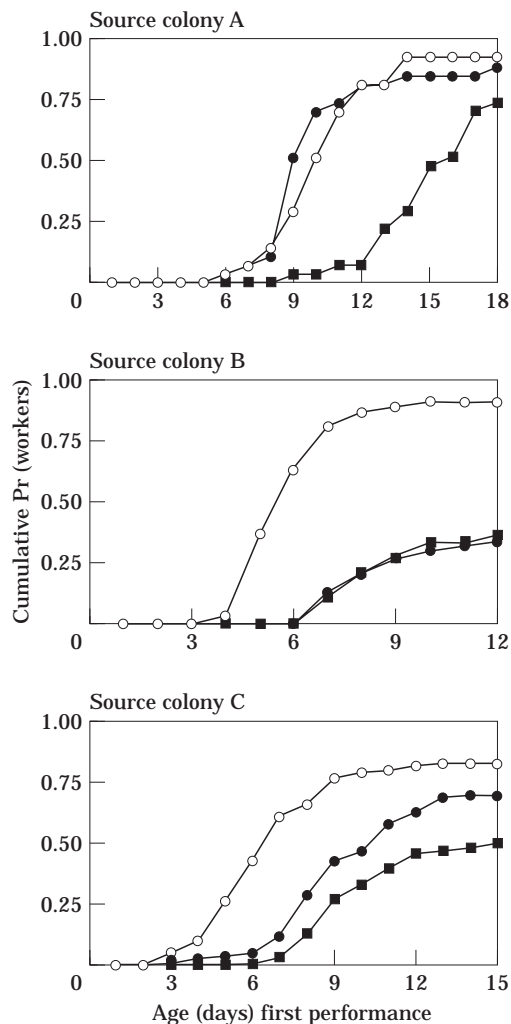
### Observation Colonies and Subject Workers

The observation colony to which workers from source colonies A and B were introduced comprised 991 unmarked adults, and the colony into which workers from source colony C were introduced comprised 633 unmarked adults when collected. Observation colonies' nests were similar in size, each containing six layers of comb. Acceptance rates of subject workers into the observation colonies were high. Of the workers introduced to the observation colonies, behavioural data were later collected on 39 (80%) from source colony A, 65 (93%) from source colony B and 170 (89%) from source colony C. The remaining workers may not have survived anaesthetization and marking. Introduced workers were never observed to be attacked or otherwise rejected by the observation colonies.

### Variability in Worker Behaviour

Workers followed the usual age polyethism sequence of moving from inside-nest to off-nest tasks, although source colony cohorts differed in their distributions of age of first performance among task sets (Fig. 3). Within source colonies, distributions of age of first performance differed significantly among inside-nest, outside-nest and off-nest tasks (survival analysis Wilcoxon test; source colony A:  $\chi^2_2=27.31$ ,  $P<0.001$ ; source colony B:  $\chi^2_2=121.59$ ,  $P<0.001$ ; source colony C:  $\chi^2_2=91.03$ ,  $P<0.001$ ). Individual workers varied in their rates of age polyethism (Fig. 3). For example, in source colony A, some workers began performing outside-nest tasks as early as 6 days of age, but others had not performed these tasks by 18 days of age.

Within task sets, workers differed in the acts they performed. I tested for genetic effects on performance of inside-nest tasks, because most workers from each source colony had performed tasks in this set by the end of the study (Fig. 3) and because genetic effects on inside-nest tasks had not been studied in epiponine wasps. All marked workers remaining at the end of the study had performed inside-nest tasks, but only a subset of these had performed nest-cleaning tasks (Figs 2 and 4; percentage of surviving marked workers that performed nest-cleaning: source colony A: 78%; source colony B: 66%; source colony C:

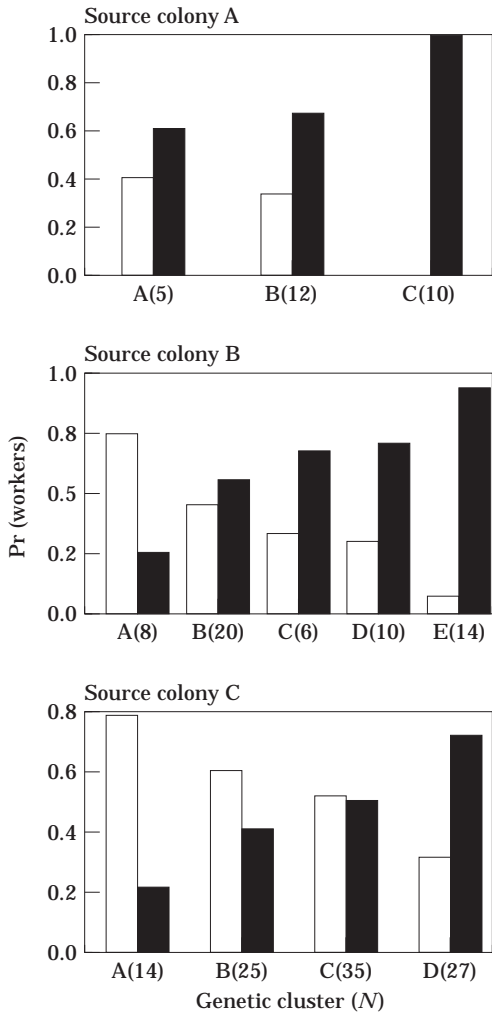


**Figure 3.** Age polyethism in *Polybia aequatorialis* workers from three colonies. The cumulative proportion of workers observed performing tasks in a given task set is plotted against age of first performance for inside-nest (○), outside-nest (●) and off-nest (■) tasks.

52%). Performance and non-performance of nest-cleaning tasks was used to differentiate workers into behavioural groups for analysis of associations of shared RAPD alleles with task performance.

### Relationship of Genetic Similarity with Rate of Age Polyethism

Of the workers introduced into the observation colonies, 27 (55%) from source colony A, 58 (83%)



**Figure 4.** Differences in task performance among clusters of genetically similar *Polybia aequatorialis* workers from three colonies. The proportion of workers that performed (■) and did not perform (□) nest-cleaning tasks is shown for each cluster of workers (number of workers in each cluster is given in parentheses).

from source colony B and 101 (53%) from source colony C remained in the observation colonies when collected and were included in analysis of RAPD allele sharing (Fig. 4). Individuals that shared RAPD alleles were not more similar in their rates of age polyethism. Survival analysis did not reveal significant associations between sharing of RAPD alleles and age of first performance of outside-nest tasks (pooled survival analysis Wilcoxon test; source colony A:  $\chi^2_{21}=21.93$ , NS;

source colony B:  $\chi^2_{22}=12.28$ , NS; source colony C:  $\chi^2_{22}=25.47$ , NS). Survival analysis also failed to support associations between shared RAPD alleles and age of first performance of off-nest foraging tasks (pooled survival analysis Wilcoxon test; source colony A:  $\chi^2_{21}=22.79$ , NS; source colony B:  $\chi^2_{22}=16.41$ , NS; source colony C:  $\chi^2_{22}=19.06$ , NS).

### Relationship of Genetic Similarity with Task Performance

The proportion of wasps that performed nest-cleaning tasks differed significantly among clusters of genotypically similar workers in the three source colonies (likelihood ratio contingency test; source colony A:  $G^2_2=6.60$ ,  $P<0.05$ ; source colony B:  $G^2_4=12.35$ ,  $P<0.05$ ; source colony C:  $G^2_3=10.43$ ,  $P<0.05$ ; Fig. 4).

## DISCUSSION

### Genetic Effects on Task Performance

The results of this study add to a growing body of evidence that individual differences in task performance include a genetic component in social insect workers (Stuart & Page 1991; Snyder 1993; Page et al. 1995b; O'Donnell 1996). In particular, genetic effects on task performance may play a significant role in structuring division of labour in polygynous wasp societies. *Polybia aequatorialis* workers that shared RAPD marker alleles tended to perform the same inside-nest tasks (this study) and off-nest foraging tasks (O'Donnell 1996). The results presented here further suggest that genetic effects on the rate of age polyethism are relatively weak or absent.

Persistent task specialization is well documented in *Polybia* spp., and flexibility in task performance may be constrained by genetic effects. *Polybia* spp. foragers rarely switch among material specializations, even in response to contingencies that change the colony's relative levels of need for different materials (O'Donnell & Jeanne 1990, 1992a, b; O'Donnell 1996). The proportions of marked, known-age *P. occidentalis* workers that foraged for nest building materials were not higher in experimentally damaged colonies than in control colonies, even though damage increased the level of colony need for building materials (O'Donnell & Jeanne 1992b). Similarly,

Jeanne (1996) showed that the main response by *P. occidentalis* foragers to alterations of colony need for materials involved changing their work rates, rather than switching among foraging tasks. In an earlier study (O'Donnell 1996), I collected most of the forager force from three *P. aequatorialis* colonies, and repeated this collection several days later on each colony. In two of the colonies, the associations between shared RAPD alleles and material specialization were the same on both collection days. In other words, the relationship between genetic and behavioural similarity remained constant within colonies, even following extreme changes in social conditions.

### Flexibility in Age Polyethism

Although workers often follow a sequence of task specializations progressing from inside to outside nest tasks, age polyethism is not a rigid developmental programme. The rate of age polyethism is flexible, changing in response to colony needs or conditions (Seeley 1982; O'Donnell & Jeanne 1992b; Robinson 1992). The present study suggests that genetic variability plays a weaker role in regulating the rate of age polyethism in *P. aequatorialis* than it plays in task specialization. In *P. occidentalis*, experimental nest damage led to a reduction of age of first foraging by workers relative to those in undamaged control colonies (O'Donnell & Jeanne 1992b). Taken together, these results indicate that environmental or social cues regulate much of the individual variation in the rate of age polyethism in epiponine wasps. The specific environmental or social cues that determine individual rates of age polyethism in *Polybia* spp. have not been identified, but they correspond to changes in colony need for performance of foraging tasks (O'Donnell & Jeanne 1992b) and may involve changes in juvenile hormone (JH) titres (O'Donnell & Jeanne 1993) as they do in honey bees (Robinson 1987; Huang et al. 1994).

Genetic effects on rate of age polyethism, as well as on differences in task performance, have been documented in honey bees (Giray & Robinson 1994; Calderone & Page 1996). This raises the question of why genetic effects on age polyethism are weak or non-existent in *P. aequatorialis*. One possible explanation lies in differences in social structure that may influence how colonies achieve efficient division of labour. *Polybia* spp. colonies are generally smaller than

*A. mellifera* colonies, with maximum populations in most species not exceeding a few thousand adults (Jeanne 1991). Honey bee colonies frequently reach populations 10 times as large (Winston 1987). Jeanne (1986) applied queuing theory to nest repair behaviour in *P. occidentalis*, and showed that both time spent in idle waiting and rates of switching among tasks were greater for workers in small colonies. Behavioural flexibility in task performance may be important in smaller societies, to minimize inefficient waiting by workers cooperating in complex tasks. Because larger colonies support greater inactive worker reserves, genetic task specialists may be less costly in larger societies: larger insect colonies may rely less on workers changing their rates of age polyethism, and more on changes in activity level by genetic task specialists.

Because they have a range of colony sizes, epiponine wasps provide subject material for a comparative test of the relationship between colony size and genetic effects on age polyethism. Average mature colony sizes of epiponines range from those much smaller than *P. aequatorialis* (several dozen adults) to those much larger (tens of thousands of adults in some *Brachygastra* spp. and *Agelaia* spp., comparable in size to honey bee colonies; Jeanne 1991). If genotypic effects on the rate of age polyethism have evolved within the epiponines, they should be best expressed in those species with very large colony sizes.

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## APPENDIX

**Sequences of PCR Primers used to Generate RAPD Marker Bands in *Polybia aquatorialis***

Primer name	Sequence (5' to 3')	Approximate size of variable marker bands in nucleotide bases*
OPA-17	GACCGCTTGT	873, 1845
OPA-18	AGGTGACCGT	234, 554, 590
OPD-06	ACCTGAACGG	381, 2460
OPD-09	CTCTGGAGAC	775
OPD-14	CTTCCCAAG	898
OPF-15	CCAGTACTCC	431
OPG-09	CTGACGTCAC	861
OPG-13	CTCTCCGCCA	431, 1353
OPG-14	GGATGAGACC	701, 849, 1046
OPI-07	CAGCGACAAG	394
OPL-13	ACCGCTGCT	1046
OPY-02	CATCGCCGCA	738, 763, 959, 1009
OPZ-11	CTCAGTCGCA	529, 677

\*Approximate size of amplified DNA fragments determined by co-migration with 123-base unit DNA molecular weight marker.

Primers were obtained from Operon Technologies, Inc.

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